

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US2004/023050

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, MEDLINE, EMBASE, WPI Data, PAJ, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>STRATIL A ET AL: "HpaII and RsaI PCR-RFLPs within an intron of the porcine leptin receptor gene (LEPR) and its linkage mapping" ANIMAL GENETICS, vol. 29, no. 5, October 1998 (1998-10), pages 405-406, XP002320727 ISSN: 0268-9146 page 406</p> <p>----- -/--</p>	<p>1,2,4, 6-8,16, 17,23, 24,30</p>

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

& document member of the same patent family

Date of the actual completion of the international search

10 March 2005

Date of mailing of the international search report

21/03/2005

Name and mailing address of the ISA

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Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

a. type of material



a sequence listing



table(s) related to the sequence listing

b. format of material



in written format



in computer readable form

c. time of filing/furnishing



contained in the international application as filed



filed together with the international application in computer readable form



furnished subsequently to this Authority for the purpose of search

2. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US2004/023050

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	VINCENT A L ET AL: "Rapid communication: A restriction fragment length polymorphism in the porcine leptin receptor (LEPR) gene" JOURNAL OF ANIMAL SCIENCE, vol. 75, no. 8, 1997, page 2287, XP002320728 ISSN: 0021-8812 the whole document	1,2,4, 6-8,16, 17,23, 24,30
X	US 6 458 531 B1 (ROTHSCHILD MAX F ET AL) 1 October 2002 (2002-10-01) claims 1-22; examples 1-4	1,2,4, 6-8,16, 17,23, 24,30
X	RUIZ-CORTES Z TATIANA ET AL: "Porcine leptin receptor: Molecular structure and expression in the ovary" MOLECULAR REPRODUCTION AND DEVELOPMENT, vol. 56, no. 4, August 2000 (2000-08), pages 465-474, XP002320729 ISSN: 1040-452X page 466; table 1 & DATABASE EMBL [Online] 29 September 1998 (1998-09-29), "Sus scrofa transmembrane leptin receptor (LEPR) mRNA, LEPR-Rb allele, complete cds." retrieved from EBI accession no. EM_PRO:AF092422 Database accession no. AF092422 C in second position = Thr (bp 221)	6-8
X	DATABASE EMBL [Online] 2 August 2000 (2000-08-02), "Sus scrofa transmembrane leptin receptor (OBR) mRNA, OBR-db allele, partial cds." XP002320733 retrieved from EBI accession no. EM_PRO:AF167719 Database accession no. AF167719 T in second position = Met (bp 189) the whole document	6-8

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International Application No

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>KORWIN-KOSSAKOWSKA AGNIESZKA ET AL: "The effect of the polymorphism of leptin (LEP), leptin receptor (LEPR) and osteopontin (OPN) genes on selected reproduction traits of synthetic Line 990 sows"</p> <p>ANIMAL SCIENCE PAPERS AND REPORTS, vol. 20, no. 3, 2002, pages 159-168, XP008044159 ISSN: 0860-4037 page 163 - page 166</p>	1,2,16, 17,23, 24,30
X	<p>SWITONSKI MAREK ET AL: "Searching for genes controlling fatness traits in pigs: A review."</p> <p>ANIMAL SCIENCE PAPERS AND REPORTS, vol. 21, no. 2, 2003, pages 73-86, XP008044189 ISSN: 0860-4037 page 78 - page 79</p>	1,2,16, 17,23, 24,30
X	<p>DATABASE BIOSIS [Online] BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; 2000, KORWIN-KOSSAKOWSKA AGNIESZKA: "[Genes for reproductive traits in pigs: A review]" XP002320732 Database accession no. PREV200100323124 abstract & PRACE I MATERIAŁY ZOOTECHNICZNE, no. 57, 2000, pages 25-37, ISSN: 0137-1649</p>	1,2,16, 17,23, 24,30

Information on patent family members

PCT/US2004/023050

Form PCT/ISA/210 (patent family annex) (January 2004)

AMENDED CLAIMS

[received by the International Bureau on 06 May 2005 (06.05.2005);
Claims 2, 3, 10, 12, 13, 15, 19, 20 and 30 are cancelled,
Claims 1, 6, 7, 9, 11, 16, 17, 21-25 and 29 are amended,
Claims 4, 5, 8, 14, 18 and 26-28 are unchanged]

1. A method of genotyping one or more animals for selecting traits capable of modulating product quality and/or productivity comprising:
 - a) obtaining a biological sample from at least one animal;
 - b) detecting at least one polymorphism in the porcine leptin receptor (pLEPR) gene; wherein the polymorphism causes a polymorphism in the pLEPR protein, wherein the polymorphism is a threonine/methionine polymorphism at amino acid number 69 of the prepro-pLEPR; and
 - c) establishing the genotype of the animal from which each biological sample was obtained and
 - d) selecting the animal having the genotype to provide the selected traitwherein the traits are selected from one or more of the group consisting of average feed intake, average daily weight gain, muscle mass, back fat, water holding capacity, meat color, meat pH, intramuscular fat, meat tenderness, and/or cooking loss.
4. The method of claim 1 wherein the presence or absence of the polymorphism is determined by a method selected from the group consisting of: DNA sequencing, restriction fragment length polymorphism (RFLP) analysis, heteroduplex analysis, single strand conformational polymorphism (SSCP) analysis, denaturing gradient gel electrophoresis (DGGE), real time PCR analysis (TAQMAN®), temperature gradient gel electrophoresis (TGGE), primer extension, allele-specific hybridization, INVADER® genetic analysis assays, and immunoassay.
5. The method of claim 1 wherein the pLEPR gene polymorphism results from the presence of either a thymidine (T) or a cytidine (C) in the second position of the codon encoding amino acid number 69 of the prepro pLEPR protein.

6. A kit for detecting the nature of a polymorphism in the porcine leptin receptor (pLEPR) gene or gene product; wherein the polymorphism produces either a threonine or a methionine at amino acid number 69 of the prepro-pLEPR protein the kit comprising a means for detecting the polymorphism in the DNA or RNA wherein the kit comprises an oligonucleotide suitable for use as a DNA or RNA probe, an oligonucleotide suitable for use as a primer in DNA or RNA synthesis; and wherein the oligonucleotide has a sequence selected from the group consisting of SEQ ID NO:1, 2, and 4-9.
7. The kit of claim 6 whereby the polymorphism is detected by one or more of the following means of detection: DNA sequencing, restriction fragment length polymorphism (RFLP) analysis, heteroduplex analysis, single strand conformational polymorphism (SSCP), denaturing gradient gel electrophoresis (DGGE), polymerase chain reaction (PCR), real time PCR analysis (TAQMAN®), and temperature gradient gel electrophoresis (TGGE); wherein the kit comprises one or more of the following: a restriction endonuclease enzyme, a DNA polymerase, a reverse transcriptase, a buffer, and deoxyribonucleotides.
8. The kit of claim 7 wherein the DNA polymerase enzyme and/or reverse transcriptase enzyme are thermostable.
9. An oligonucleotide that comprises a sequence selected from the group selected from SEQ ID NO:1, 2, and 4-9.
11. The oligonucleotide of claim 9 which has a sequence selected from the group consisting of SEQ ID NO:1, 2, and 4-9.

14. A method of enhancing a trait selected from the group consisting of: average feed intake and/or average daily weight gain, backfat, muscle mass, water holding capacity, meat color, meat pH, intramuscular fat, meat tenderness, and/or cooking loss of animals in a pig herd, the method comprising:
- a) screening a plurality of pigs to identify the nature of an allelic variant in the porcine Leptin receptor (pLEPR) gene, wherein said allelic variant produces a threonine or methionine polymorphism at amino acid number 69 of the prepro-pLEPR protein;
 - b) selecting those pigs having a desired allele;
 - c) using the selected pigs as sires/dams in a breeding plan to produce offspring; wherein the offspring have an increase frequency of the desired allele.
16. A pig offspring produced by a method for enhancing a trait selected from the group consisting of: average feed intake and/or average daily weight gain, backfat, muscle mass, water holding capacity, meat color, meat pH, intramuscular fat, meat tenderness, and/or cooking loss of animals in a pig herd, the method comprising:
- a) screening a plurality of pigs to identify the nature of an allelic variant in the porcine leptin receptor (pLEPR) gene, wherein said allelic variant produces a threonine or methionine polymorphism at amino acid number 69 of the prepro-pLEPR protein;
 - b) selecting those pigs having a desired allele;
 - c) using the selected pigs as sires/dams in a breeding plan to produce offspring; wherein the offspring have an increase frequency of the desired allele.

17. A pig herd having an altered frequency of a specific allele of the porcine leptin receptor (pLEPR) gene; the allele selected for modifying a trait selected from the group consisting of: average feed intake and/or average daily weight gain, backfat, muscle mass, water holding capacity, meat color, meat pH, intramuscular fat, meat tenderness, and/or cooking loss, wherein the herd is produced by a method comprising:
 - a) screening a plurality of pigs to identify the nature of an allelic variant in the porcine leptin receptor (pLEPR) gene, wherein said allelic variant produces a threonine or methionine polymorphism at amino acid number 69 of the prepro-pLEPR protein;
 - b) selecting those pigs having a desired allele;
 - c) using the selected pigs as sires/dams in a breeding plan to produce, as offspring, a second plurality of pigs; wherein the offspring have an altered frequency of the desired allele;
 - d) repeating steps a) through c) until an altered allelic frequency is achieved.
18. A method of enhancing meat production from a swine herd comprising:
 - a) screening a plurality of pigs to identify the nature of an allelic variant in the porcine leptin receptor (pLEPR) gene, wherein said allelic variant produces a threonine or methionine polymorphism at amino acid number 69 of the prepro-pLEPR protein;
 - b) selecting those pigs having a desired allele;
 - c) using the selected pigs as sires/dams in a breeding plan to produce offspring; wherein the offspring have an increase frequency of the desired allele;
 - d) repeating steps a) through c) until an increased allelic frequency for the desired allele is achieved.
21. The method of claim 17 wherein the frequency of the allele is decreased in the second plurality of pigs as compared with the frequency of the allele in the first plurality of pigs.

22. The method of claim 17 wherein the frequency of the allele is increased in the second plurality of pigs as compared with the frequency of the allele in the first plurality of pigs.
23. A pig population produced by the method of claim 17.
24. Offspring produced by the method of claim 17.
25. A method of identifying a polymorphism that modifies a trait selected from the group consisting of: average feed intake and/or average daily weight gain, backfat, muscle mass, water holding capacity, meat color, meat pH, intramuscular fat, meat tenderness, and/or cooking loss by identifying a single nucleotide polymorphism in linkage disequilibrium with the threonine/methionine polymorphism at amino acid 69 of the prepro porcine leptin receptor (pLEPR) protein (T69M polymorphism), the method comprising:
 - a) identifying at least one large-insert genomic clone containing all or a portion of the pLEPR gene;
 - b) determining the sequences of all or a portion of the clone(s);
 - c) identifying target regions in close proximity to the pLEPR gene;
 - d) screening a panel of animals to determine the sequence of the target regions;
 - e) identifying any single nucleotide polymorphisms (SNPs) present in the target regions to provide a set of at least one target SNP;
 - f) determine which of the target SNPs is in linkage disequilibrium with the T69M polymorphism.
26. The method of claim 25 wherein the large-insert genomic clone is selected from the group consisting of a bacterial artificial chromosome (BAC), yeast artificial chromosome (YAC), P1 phage, cosmid, fosmid, phage, or plasmid constructs.
27. The method of claim 25 wherein the sequence of the clone is determined by a method comprising a of polymerase chain reaction amplification of a portion of the clone.

28. The method of claim 25 where the identified target regions are within 5 centiMorgans or 5 million base pairs of the pLEPR gene.
29. The method of claim 18 wherein part b) further comprises:
- b') tabulating the identified nature of the allelic variance possessed by each pig;
and
 - b'') utilizing the tabulated variances as part of a program of marker assisted selection and/or marker assisted allocation.